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Urinary allantoin excretion can be used as an estimate of microbial crude protein supply.

Summary

Two metabolism trials evaluated urinary allantoin excretion as a noninvasive marker of microbial crude protein flow from the rumen. In the first trial, both urinary allantoin excretion and duodenal purine flow increased as alfalfa intake increased. A positive linear relationship (r^2 = .74) existed between the markers. In the second trial, an increase in the metabolizable energy supplied to the animal did not increase urinary allantoin excretion. We concluded that urinary allantoin excretion was an effective, noninvasive marker of microbial crude protein supply.

Introduction

A urine metabolite, allantoin, has received recent attention in dairy and sheep research for its potential to serve as a microbial crude protein (MCP) marker. No validation studies have been conducted that compare urinary allantoin excretion (ALLAN) to duodenal flow of MCP for beef cattle diets. Such a comparison would provide a benchmark for the use of ALLAN in field trials. It may be inaccurate to assume ALLAN is the same for all dietary conditions. The objectives of the research reported herein were: 1) to compare ALLAN to the duodenal flow of MCP measured by purines; 2) to describe the effect of increasing alfalfa hay intake on the variables in objective 1); and 3) to investigate effects of metabolizable energy supply on ALLAN.

Procedure

Six crossbred yearling steers (mean wt = 800 lb.) were fitted with ruminal and duodenal cannulae according to the guidelines of the UNL Institutional Animal Care and Use Committee. The diet fed in two metabolism trials was a single lot of alfalfa hay (DM basis): 22.4% CP, 32.8% NDF, and 26.7% ADF. A pre-trial feeding period determined the maximum level of dry matter intake that all steers would sustain without any feed refusal was 19 lb/day. In Trial 1, fractions of the maximum level were applied as treatments in a concurrent 3 x 3 Latin square arrangement: 1) 9.5 lb/day; 2) 14.3 lb/day; or 3) 19 lb/day. Periods were 21 days. Hay was fed every 2 hours by automatic feeders in an attempt to establish a steady state of fermentation in the rumen. Steers were housed in 10' x 10' box stalls and were allowed to move freely on days 1-14 of each period. On days 15-21, steers were tethered to facilitate sample collection. Duodenal samples were collected every 3 hr from 0700 to 1900 on days 15-17 and total urine collection was made on days 18-21 by abdominal funnels attached to a vacuum pump. Duodenal samples were freeze-dried and analyzed for purines as a microbial marker and acid-insoluble ash as a flow marker. Aliquots of each total urine collection were analyzed for allantoin.

Trial 2 was conducted as a follow-up to Trial 1. The following two treatments were applied over six-day periods in a crossover design: 1) 14.3 lb/day of alfalfa hay DMI plus ruminal infusion of isotonic saline; or 2) 14.3 lb/day of alfalfa hay DMI plus ruminal infusion of a sodium acetate and propionic acid solution (pH = 6.0) formulated to provide the metabolizable energy equivalent of 4.7 lb of alfalfa hay DMI. The ruminal infusions were given as pulse doses through the ruminal cannula four times daily. Cattle were tethered on days 4-6 and total urine collections were made at that time. Aliquots of urine were analyzed for allantoin.

Results

A direct comparison of the daily supplies of purines and allantoin (Figure 1) indicates there is a linear relationship between them. The fit of the line (as described by the r^2 value) implies the line does an adequate job of describing the variation between the two methods. An r^2 value of .74 can be interpreted as the line describing 74% of the variation in the data. The fact that the slope of the line (.84) is less than one indicates that not all of the daily purine flow at the duodenum is recovered in the urine as allantoin. Slopes of less than one for comparisons between post-ruminal purine flow and ALLAN have been reported in the scientific literature. The possibility exists for purines to be metabolized to derivatives other than allantoin and excreted in the urine.

The effect of alfalfa DMI on the supply of each MCP marker is shown in Figure 2. The upward slope of the regression lines for both purine and allantoin indicates an increase in alfalfa intake caused an increase in MCP marker supply. This is intuitively correct; an

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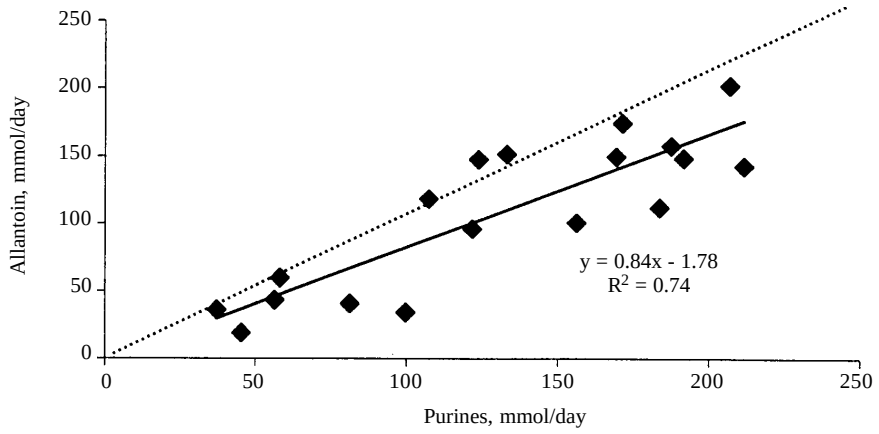

Figure 1. Comparison of duodenal purine flow and urinary allantoin excretion in beef cattle. The dotted line represents 100% recovery of purines as urinary allantoin. The solid line represents the actual recovery of purines as allantoin (equation for solid line shown on graph).

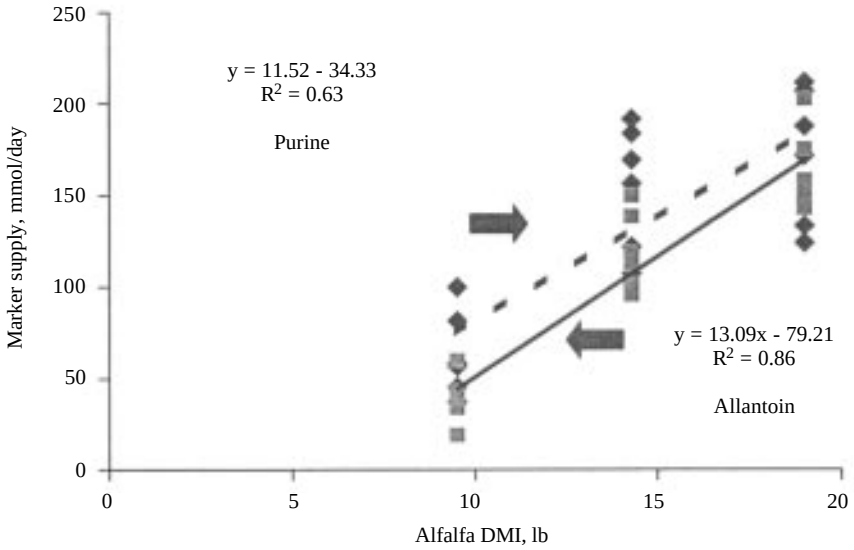

Figure 2. Effect of alfalfa dry matter intake on microbial crude protein marker supply.

increase in the TDN available for rumen fermentation should increase the amount of microbial cells and constitutive MCP markers. The degree of fit for each regression equation is also shown. The higher r^2 value for allantoin (.86) indicates it does a better job of describing the increase in MCP supply caused by additional alfalfa DMI than the does the purine assay (.63). This may be because the compound errors associated with the measurement of purine supply in duodenal samples lead to more variation in those estimates. Both an MCP marker (purine) and a duodenal flow marker (acid-insoluble ash, in this trial) are incorporated into a duodenal supply estimate, whereas a urine excretion estimate is based only on one marker (allantoin).

There was no difference in the urinary excretion of allantoin for the treatments imposed in Trial 2 (data not shown). The overall mean allantoin excretion (mmol/day) for Trial 2 was 128.3 mmol/day. This value is similar to the mean allantoin excretion (119.7 mmol/day) for the cattle fed the same level of alfalfa DMI in Trial 1. The experimental infusion of volatile fatty acids in Trial 2 was designed to provide the metabolizable energy equivalent of 4.7 lb of alfalfa DMI without allowing the rumen bacteria access to the energy. The hypothesis being tested was whether or not the additional metabolizable energy provided to the animal by the added alfalfa increased the endogenous excretion of allantoin. We conclude metabolizable energy level has no effect on endogenous allantoin excretion and all the increase in allantoin exhibited by increased alfalfa DMI in Trial 1 is due to rumen microbes. Allantoin is the product of hepatic oxidation of purines and is cleared by the kidney at a rapid rate. Intermediate products of this oxidation (such as xanthine and uric acid) do occur in the urine, but they are a small fraction of the total oxidized purines (a.k.a. purine derivatives), so we did not analyze for them. Another reason we did not analyze for the other purine derivatives is that our assay is only specific for allantoin. While other assays exist that do measure all the purine derivatives, they are more complex to perform.

We conclude there is a strong linear relationship between duodenal purine flow and urinary allantoin excretion. This relationship allows the use of allantoin as a noninvasive marker of MCP supply. Urinary allantoin excretion is a simpler method of estimating MCP supply than the duodenal purine method. Further research will focus on describing the relationship between these MCP markers and the actual amount of microbial crude protein they represent.

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